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Reemergence of Gentamicin-Susceptible Strains of Methicillin-Resistant *Staphylococcus aureus* in France: a Phylogenetic Approach

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The reemergence of gentamicin-susceptible (Gen^s) methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in France between 1992 and 1996 was investigated using a phylogenetic approach (multiprimer randomly amplified polymorphic DNA typing). Eighty-six percent (65 of 85) of the French strains were grouped into one phylogenetic cluster within which all but one Gen^s strain were grouped into a subcluster. Thus, the reemergence of Gen^s MRSA strains in France was likely due to the spread of one specific clone which belonged to a cluster comprising most French gentamicin-resistant (Gen^r) strains. This suggests that the Gen^s clone has emerged from a Gen^r strain of this cluster.

In France, the proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) among *S. aureus* strains, ranging from 30 to 40%, is one of the highest in Europe (13). In addition, the resistance of MRSA to multiple aminoglycosides, including resistance to gentamicin (Gen^r), was over 90% in the beginning of the 1990s. Since 1992, several hospitals have observed the reemergence of strains which were susceptible to gentamicin (Gen^s) (1, 10, 11; L. Gazagne, P. Guedet, and E. Lecaillon, Abstr. 5th Int. Conf. Prev. Infect., abstr. MID/AMR 02, 1998; P. Guedet, L. Gazagne, E. Lecaillon, A. Le Coustumier, R. Bismuth, and "Le College de Bacteriologie, Virologie et Hygiene des Hopitaux de France," Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-122, 1997). These strains were also characterized by the unexpected reappearance of heterogeneous resistance to oxacillin and variable resistance to other antibiotics. It was speculated that changes in the use of antibiotics in French hospitals, especially the decreased consumption of aminoglycosides, could be responsible for the emergence and spread of Gen^s MRSA strains.

Unlike the situation in Belgium, where the emergence of Gen^s strains was due to a new MRSA clone (5, 6), it was speculated that French Gen^s strains emerged from Gen^r MRSA by means of the loss of the *aac6′-aph2″* gene, which confers resistance to all aminoglycosides, with conservation of the *ant4′* gene conferring resistance to kanamycin, tobramycin, and amikacin (10). Since the *aac6′-aph2″* gene is frequently carried by transposon Tn*4001* (4, 8), it is likely that certain Gen^s MRSA strains could have emerged from Gen^r MRSA strains by means of transposon excision or deletion. This hypothesis is supported by the fact that (i) both Gen^r and Gen^s strains have closely related pulsed-field gel electrophoresis (PFGE) types, which suggests a recent common ancestor, and (ii) in vitro long-term storage of Gen^r MRSA at room temperature may result in spontaneously occuring Gen^s MRSA derivatives (10).

Whereas one report using molecular typing data (PFGE) indicated that one major clone type was responsible for most Gen^s isolates (1), other reports identified two to three major types (7, 10, 11). As PFGE typing of MRSA is a highly discriminatory method, markers might evolve very rapidly and the evaluation of older genetic relationships is therefore difficult.

The aim of the present study was to further investigate this issue by a population genetics analysis of both Gen^r and Gen^s strains isolated in France during the last decade in order to (i) determine if Gen^s strains in France were derived from a common or different ancestor(s) and (ii) confirm that this ancestor(s) was phylogenetically related or not related to Gen^r strains. We used a typing method, multiprimer randomly amplified polymorphic DNA (RAPD), which was validated to produce information on the phylogenetic relationships between isolates (2).

Seventy-five French MRSA strains were randomly selected from three sources: (i) 11 Gen^r and 11 Gen^s strains of MRSA from a study which included 895 isolates collected from 96 nonuniversity French hospitals in 1995 (Guedet et al., 37th ICAAC), (ii) 9 Gen^r and 13 Gen^s strains of MRSA isolated in 1997 to 1998 from the hospital of Perpignan, France (Gazagne et al., Abstr. 5th Int. Conf. Prev. Infect.), and (iii) 12 Gen^r and 8 Gen^s strains isolated in 1996 and 11 Gen^r strains isolated in 1990 from the National Reference Center for Staphylococcal Toxemia in Lyon, France. In addition, 24 strains isolated from other European countries were selected from a previous study and are representative of the diversity of MRSA observed (2).

Multiprimer RAPD typing consists of using the RAPD technique with 10 consecutively selected primers, as already described (2). In brief, DNA was extracted, purified, and quantified before its use for the PCR. Twenty nanograms of template DNA was used in each PCR. The following primers were used separately: A-03 (AGTCAGCCAC), A-13 (CAGC

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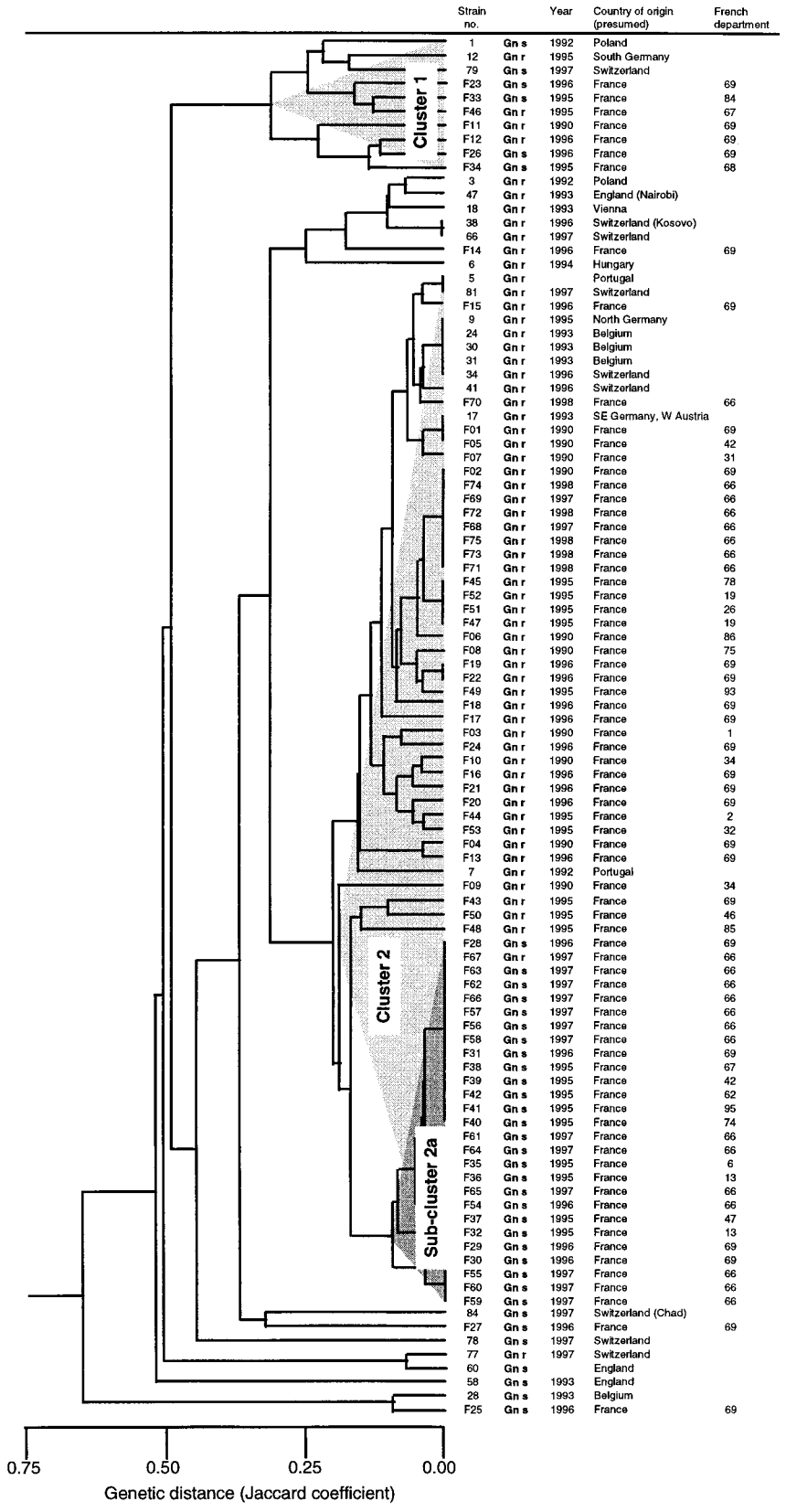

FIG. 1. Dendrogram (derived using the unweighted pair-group method with arithmetic averages) based on the Jaccard's distance matrix of RAPD results from 100 MRSA strains (75 from France and 25 from other European countries). The susceptibilities to gentamicin (Gen^s and Gen^r are represented as Gn s and Gn r) and the year, country, and department of France in which each MRSA strain was isolated are indicated.

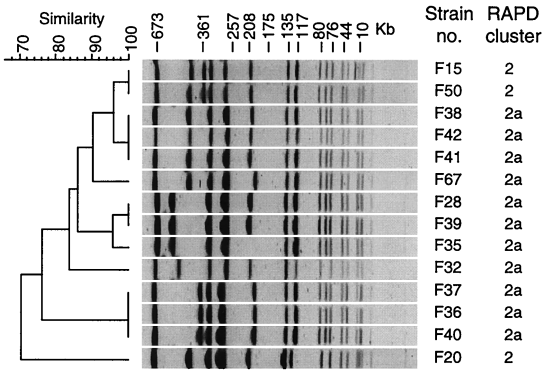

FIG. 2. PFGE typing of Gen^s and Gen^r strains from RAPD clusters 2 and 2a. The Dice coefficient was used to calculate the similarity between each pair of strains.

ACCCAC), A-17 (GACCGCTTGT), B-05 (TGCGCCCTTC), B-15 (GGAGGGTGTT), F-04 (GGTGATCAGG), N-10 (ACAACTGGGG), N-15 (CAGCGACTGT), R-07 (ACTGGCCTGA), R-20 (ACGGCAAGGA), U-08 (GGCGAAGGTT), and U-19 (GTCAGTGCGG). The amplified products were separated by electrophoresis in agarose gel and stained with ethidium bromide. The Jaccard's distance was used to estimate the genetic difference between each pair of isolates. The unweighted pair-group method with arithmetic averages was used as a clustering method.

RAPD results from the 75 French MRSA strains and the 24 strains from other European countries with the 10 primers were analyzed, and the phylogenetic relatedness between these strains is shown in the dendrogram of Fig. 1. Most of the French strains were distributed into two clusters of 65 and 7 strains, respectively. Both clusters comprised Gen^s and Gen^r strains. The smaller cluster, cluster 1, included three Gen^r and four Gen^s strains as well as some other European Gen^r and Gen^s strains. The larger cluster, cluster 2, included epidemic clones of Gen^r MRSA from all over Europe, as well as 39 Gen^r and 26 Gen^s strains from France. However, within this cluster, a subcluster showing a relative genetic homogeneity compared to other strains of the cluster could be clearly identified (Fig. 1). This subcluster, labeled 2a, includes all but one French Gen^s strain. Among the 27 French hospitals from which MRSA of this study was recovered, strains of cluster 2 Gen^r and Gen^s were found in 25 different hospitals distributed all over the territory of France; Gen^r strains were found in 16 hospitals, and Gen^s strains were found in 10 hospitals. Other Gen^s strains were found in only three hospitals.

Results of multiprimer RAPD typing showed that most of the French MRSA belonged to the same phylogenetic cluster, which comprised both Gen^r and Gen^s strains. The fact that the Gen^s strains were closely related suggests that their emergence in France was mainly due to the spread of one specific clone. Moreover, the fact that this Gen^s clone belongs to a phylogenetic cluster originally comprising only Gen^r strains strongly supports the hypothesis that it emerged from a Gen^r strain, a situation which is different from the one reported in Belgium, where Gen^s strains are due to the emergence of a new clone represented by strain 28 shown in Fig. 1 (5, 6).

PFGE typing has been performed on 11 strains of cluster 2a (1 strain per French institution) and 3 Gen^r strains of cluster 2 (Fig. 2). A one-band difference was observed between some Gen^s and Gen^r strains (e.g., strains F38 and F50), confirming previously published results (10). However, the close genetic relationship between all strains of cluster 2a, as demonstrated by RAPD, was more difficult to assess with PFGE, since up to seven-band differences were observed between some strains (e.g., F32 and F37) (Fig. 2). For strains belonging to cluster 2, according to RAPD, up to a nine-band difference was observed with PFGE (between strains F20 and F40) (Fig. 2). If standard interpretation criteria for PFGE (12) were used, these strains would be considered unrelated. These results suggest that the markers of PFGE probably evolved more rapidly than those of multiprimer RAPD and thus are less suitable for evaluation of older genetic relationships.

The decrease in aminoglycoside use in French hospitals was suggested as the cause of the emergence and spread of Gen^s MRSA strains (1, 7). However, there are three pieces of evidence that this reduction might not be the only factor: (i) the diminution of aminoglycoside use would favor an increase of other Gen^s strains (e.g., strains of cluster 1) instead of only one clone, (ii) this decrease in use has not been reported in all hospitals, and (iii) it does not explain the increase in susceptibility to other antibiotics (10). The rapid spread of this clone does not seem to be attributable to poor infection control in French hospitals because, in most of them, policies have been reinforced during the last decade. It is likelier that this clone has acquired a fitness advantage during its evolution. In vitro experiments showed that the growth rate of Gen^s isolates of this clone was higher than that of the Gen^r strains (9), a finding consistent with a fitness advantage of Gen^s isolates. In addition, this new Gen^s clone was characterized by the reappearance of heterogeneous resistance to oxacillin, a characteristic similar to that found in the epidemic Belgium clone 2, which was also characterized by rapid spreading (3). Thus, the emergence and rapid spread of this new Gen^s clone support the hypothesis that epidemic clones emerge after the acquisition through mutations of some fitness advantage over the other clones. The loss of this fitness advantage during the microevolution of the clone would predict its fading.

In conclusion, the reemergence of Gen^s MRSA strains in France is likeliest due to the spread of one epidemic clone. This clone belongs to the same phylogenetic cluster that includes most Gen^r MRSA strains recovered in France, which adds to the evidence that it is derived from a Gen^r strain.

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